

2017-01-03

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Green, DS

<http://hdl.handle.net/10026.1/8161>

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10.1021/acs.est.6b04496

Environmental science & technology

American Chemical Society (ACS)

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1 'This is a copy of the accepted paper as submitted for final publication. The final published version  
2 can be found at <http://pubs.acs.org/doi/abs/10.1021/acs.est.6b04496>

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**Microplastics affect the ecological functioning of an important biogenic habitat**

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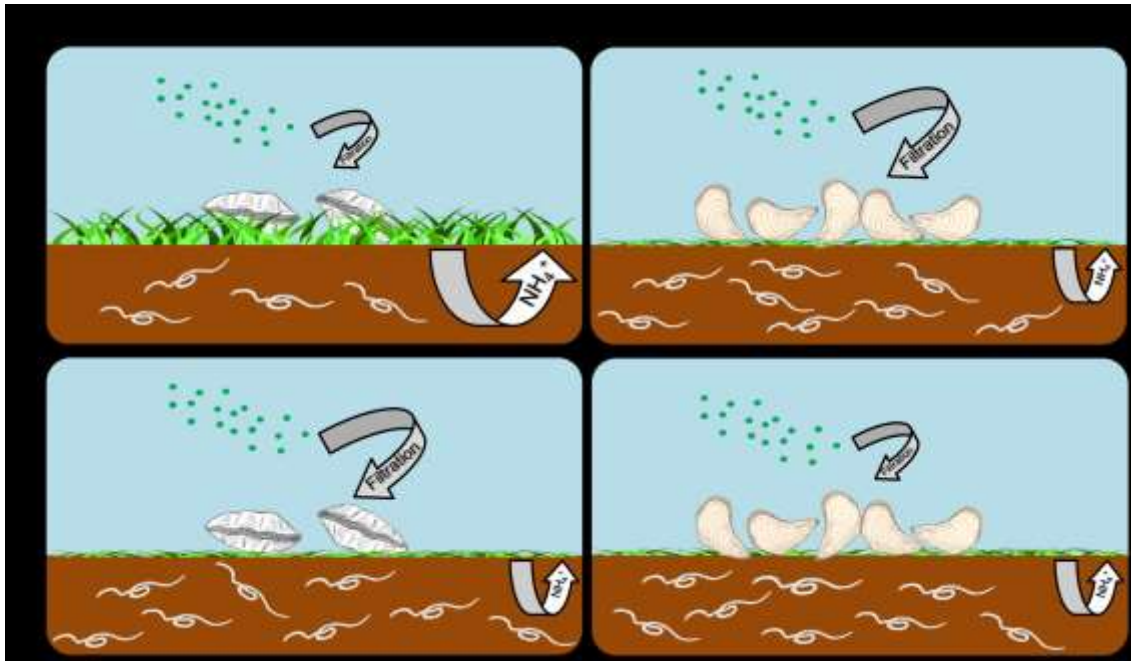
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**Keywords:** plastic pollution, *Ostrea edulis*, *Mytilus edulis*, polyethylene, polylactic acid, biogeochemistry, nutrient cycling

## 25 Abstract

26 Biological effects of microplastics on the health of bivalves have been demonstrated  
27 elsewhere, but ecological impacts on the biodiversity and ecosystem functioning of bivalve-  
28 dominated habitats are unknown. Thus, we exposed intact sediment cores containing  
29 European flat oysters (*Ostrea edulis*) or blue mussels (*Mytilus edulis*) in seawater to two  
30 different densities (2.5 or 25  $\mu\text{g L}^{-1}$ ) of biodegradable or conventional microplastics in  
31 outdoor mesocosms. We hypothesised that filtration rates of the bivalves, inorganic nitrogen  
32 cycling, primary productivity of sediment dwelling microphytobenthos, and the structure of  
33 invertebrate benthic assemblages would be influenced by microplastics. After 50 days,  
34 filtration by *M. edulis* was significantly less when exposed to 25  $\mu\text{g L}^{-1}$  of either type of  
35 microplastics, but there were no effects on ecosystem functioning or the associated  
36 invertebrate assemblages. Contrastingly, filtration by *O. edulis* significantly increased when  
37 exposed to 2.5 or 25  $\mu\text{g L}^{-1}$  of microplastics, and porewater ammonium and biomass of  
38 benthic cyanobacteria decreased. Additionally the associated infaunal invertebrate  
39 assemblages differed, with significantly less polychaetes and more oligochaetes in treatments  
40 exposed to microplastics. These findings highlight the potential of microplastics to impact the  
41 functioning and structure of sedimentary habitats and show that such effects may depend on  
42 the dominant bivalve present.

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## 45 **Introduction**

46 Microplastics contaminate marine habitats across the globe<sup>1</sup> and are recognised as a  
47 significant environmental challenge requiring urgent management<sup>2</sup>. It has recently been  
48 suggested that they are the most abundant form of solid waste on Earth<sup>1</sup> and their abundance  
49 is increasing<sup>3</sup>. Although there is much uncertainty regarding the concentrations of  
50 microplastics in the environment, high concentrations in seawater of  $\sim 3\text{-}23\ \mu\text{g L}^{-1}$  and even  
51 up to  $\sim 4500\ \mu\text{g L}^{-1}$  have been reported<sup>4,5,6</sup> in some heavily contaminated areas. Despite this  
52 prevalence, their effects on marine ecosystems are not well understood. Research to date has  
53 mostly focused on effects of microplastics on individual species, but effects on assemblages  
54 and ecosystem functioning within coastal habitats remain largely unknown<sup>7,8</sup>.

55 Previous research has concentrated on organisms that ingest microplastics directly, such as  
56 filter-feeders, including marine mussels<sup>9,10,11</sup> and oysters<sup>12,13</sup>. These organisms are typically  
57 chosen for exposure experiments due to their great filtration capacity. For example,  
58 individual mussels and oysters can filter  $\sim 0.5\text{-}2.5$ <sup>14</sup> and  $\sim 5\text{-}25$ <sup>15</sup> L of seawater h<sup>-1</sup>  
59 respectively. As such, they are very likely to ingest microplastics<sup>16</sup>, and indeed, specimens  
60 from the field have been found to contain microplastics<sup>11,17,18</sup>. Exposure to relatively high  
61 densities of microplastics has been found to alter the respiration rates<sup>13</sup>, immunology<sup>10</sup>,  
62 reproductive capacity and filtration rates<sup>12</sup> of bivalves. Owing to their role as ecosystem  
63 engineers, such effects are likely to permeate beyond the individual organism. For example,  
64 reefs created by mussels and oysters provide refugia and nursery grounds for other,  
65 commercially-important species and can support diverse communities<sup>19,20</sup>. In addition, filter  
66 feeding leads to benthic-pelagic coupling; channelling nutrients from the water column and  
67 locally concentrating them via biodeposition (i.e. deposition of faeces and pseudo-faeces).  
68 Mussels and oysters can, therefore, enhance the release of limiting inorganic nutrients, such  
69 as ammonium, from sediments, fuelling primary productivity of the microphytobenthos (such

70 as diatoms and cyanobacteria) in the sediment, which in turn supports benthic and pelagic  
71 food webs<sup>21</sup>. If microplastics alter the ability of these organisms to filter feed, there may be  
72 wider impacts on their associated communities and on the functioning of coastal ecosystems.  
73 In addition, biodeposition is a likely mechanism by which suspended microplastics are  
74 transported from the pelagic zone onto sediments<sup>22</sup>. Mussels and oysters may, therefore,  
75 locally concentrate microplastics potentially altering biogeochemical processes, the biomass  
76 of primary producers and macrofaunal assemblages within the sediment.

77 In response to concerns of globally increasing plastic pollution, demand for biodegradable  
78 plastics has risen, with annual global production predicted to quadruple over the next five  
79 years<sup>23</sup>. It is thought that the replacement of conventional plastics, such as high density  
80 polyethylene (HDPE), with biodegradable alternatives, such as polylactic acid (PLA) will  
81 reduce the persistence, and therefore the impacts, of plastic pollution<sup>24</sup>. However, methods  
82 developed to assess the rate and extent of biodegradability of plastics in marine environments  
83 (e.g. ASTM International D7991-15)<sup>25</sup> are still limited in their ability to predict degradation  
84 in natural habitats<sup>26</sup>. The potential for PLA and other bioplastics, or biodegradable plastics to  
85 persist as microscopic particles, or to affect assemblages of organisms in the marine  
86 environment before they degrade, remains largely unknown. Recently, however, Green,  
87 (2016)<sup>13</sup> showed that PLA microplastics can lead to alterations in assemblage structure of  
88 macrofauna in sandy sediment with oysters.

89 In this study the effects of microplastics composed of HDPE or PLA, at two densities, on the  
90 structure and functioning of bivalve-dominated habitats were assessed using intact sediment  
91 cores in outdoor mesocosms, providing controlled, semi-natural conditions. Two experiments  
92 were conducted using two common, filter-feeding bivalves; blue mussels (*Mytilus edulis*) and  
93 European flat oysters (*Ostrea edulis*). The experiments tested the hypotheses that repeated  
94 exposure to biodegradable (PLA) and conventional (HDPE) microplastics in the water

column would alter the: (i) filtration rates of the bivalves; (ii) concentration and fluxes of benthic inorganic nitrogen; (iii) biomass of benthic micro-algae; and (iv) diversity and abundance of macrofauna within sedimentary habitats associated with either species of bivalve.

## 2. Methodology

### 2.1. Experimental design and set-up

Two separate mesocosm experiments, one focusing on *M. edulis* and one on *O. edulis* habitats, were set up simultaneously at the outdoor flow-through mesocosm facility at Queen's University Marine Laboratory, Portaferry, Northern Ireland. Both experiments had the same asymmetric design, with two fixed, orthogonal factors: "Plastic", with two levels: polylactic acid (PLA) and high density polyethylene (HDPE) and "Dose", with two levels: 2.5  $\mu\text{g L}^{-1}$  and 25  $\mu\text{g L}^{-1}$  seawater. A single treatment, without any added microplastics, was used as a control. To estimate the densities of microplastics in each treatment, water samples were taken from each "Plastic x Dose" treatment on days 1, 26 and 48 and microplastic particles were counted using a haemocytometer (Table S1). For each experiment, all treatments were replicated five times ( $n = 5$ ,  $N = 25$  per species) for a total of 50 mesocosms. Although the applied doses were relatively high compared with average densities observed and reported in the literature from  $\sim 330 \mu\text{m}$  plankton-net tow samples<sup>27</sup>, at smaller mesh sizes (50  $\mu\text{m}$ ) densities of up to 7800 particles  $\text{L}^{-1}$  (equating to  $\sim 4500 \mu\text{g L}^{-1}$ ) have been found in heavily contaminated coastal waters<sup>6</sup>. Furthermore, the densities used in the current study are among the lowest used experimentally to date<sup>28</sup> and were chosen to approximately reflect high values currently (2.5  $\mu\text{g L}^{-1}$ ) and in the future (25  $\mu\text{g L}^{-1}$ ) based on the prediction that global plastic waste input will increase 10-fold by 2025<sup>27</sup>.



119 The mesocosms were made using clean, opaque 10 L polypropylene buckets (height x  
 120 diameter = 25 x 25 cm), placed onto large basins (as shown in Green 2016<sup>13</sup>). Each  
 121 mesocosm had an overflow pipe, allowing drainage directly into the basin. Waste water did  
 122 not come into contact with other mesocosms and each mesocosm was an independent  
 123 replicate. In order to minimize disturbance to the sediment water interface, mesocosms were  
 124 equipped with sampling ports, drilled at 0, 1 and 4 cm into the sediment (Figure S1). These  
 125 ports were plugged until required for nutrient sampling (see section 2.3).  
 126 Each mesocosm was filled up to 4 cm depth with an intact core of muddy sediment, collected  
 127 using a mesocosm with the bottom cut out, from an area (~25 x 25 m) of a nearby shore  
 128 where *M. edulis* and *O. edulis* were abundant. Sand-filtered seawater, sourced directly from  
 129 Strangford Lough (54°22'51.1"N; 5°33'04.0"W) was delivered via dedicated, individual  
 130 hoses to each mesocosm at constant flow rates (~500 mL minute<sup>-1</sup>), giving an overlying water  
 131 column of ~8 L and a daily turnover rate of 60 L day<sup>-1</sup>. The mesocosms were left to  
 132 acclimatise for 48 h before live *M. edulis* or *O. edulis* were added. *M. edulis* and *O. edulis*  
 133 were collected from the same shore as the mud and were measured, weighed and allocated  
 134 randomly to treatments in order to ensure that no biases due to size were introduced into the  
 135 experiments. The collected *M. edulis* had an initial average ( $\pm$  S.E.M.) wet biomass of  $20.1 \pm$   
 136  $1.7$  g, maximal length of  $47.9 \pm 0.6$  mm, width of  $21.5 \pm 0.4$  mm and height of  $23.5 \pm 0.3$  mm  
 137 ( $n = 175$ ). The collected *O. edulis* had an initial average ( $\pm$  S.E.M.) wet biomass of  $36.0 \pm 5.2$   
 138 g, maximal length of  $63.0 \pm 1.6$  mm, width of  $60.1 \pm 1.1$  mm and height of  $14.9 \pm 0.6$  mm ( $n$   
 139  $= 50$ ). Dimensions were measured with a calliper. On the 24<sup>th</sup> of August 2014, seven  
 140 individuals of *M. edulis* (equivalent to individuals  $142.6 \text{ m}^{-2}$ ) were placed into 25 separate  
 141 mesocosms and two individuals of *O. edulis* (equivalent to individuals  $40.7 \text{ m}^{-2}$ ) were placed  
 142 into each of the other 25 mesocosms. These densities were chosen to reflect those high  
 143 enough to be considered "*M. edulis* dominated" or "*O. edulis* dominated" habitats (i.e. > 30%

144 cover and 5 individuals m<sup>-2</sup> for *M. edulis* and *O. edulis* respectively, as defined by OSPAR<sup>29</sup>).  
 145 The bivalves were placed on the surface of the sediment to mirror how they occurred locally  
 146 in the field. There were no significant differences between the biomasses of individuals  
 147 allocated to the different treatments at the start of the experiment (one-way ANOVA based on  
 148 averaged dimensions in each mesocosm: *M. edulis*:  $F_{4,20} = 0.32$ ,  $P = 0.861$ , *O. edulis*:  $F_{4,20} =$   
 149  $0.26$ ,  $P = 0.902$ ). The microplastic particles used in the experiment were of a similar colour  
 150 (white) and size range, although their volume-weighted mean diameters differed: 65.6  $\mu\text{m}$   
 151 (range = 0.6–363  $\mu\text{m}$ ) for PLA and 102.6  $\mu\text{m}$  (range = 0.48–316  $\mu\text{m}$ ) for HDPE. In order to  
 152 introduce microplastics into the mesocosms in a realistic manner, a dietary exposure method  
 153 was used. In brief, microplastics were added to separate cultures (10 L) of the microalgae,  
 154 *Isochrysis galbana* and left for 3 days with constant aeration. This was long enough for the  
 155 microplastics to become more neutrally buoyant; i.e. move more freely within the culture  
 156 containers rather than clinging to the sides or floating on top of the water. Fresh batches of  
 157 control and microplastic dosed algae cultures were made up weekly. In order to ensure that  
 158 the concentrations of *I. galbana* did not differ between treatments, algal cells were counted  
 159 from each culture using a haemocytometer (on days 1, 26 and 48, Table S2). There were no  
 160 significant differences in the density of *I. galbana* cells between treatments (one-way  
 161 ANOVA for day 1:  $F_{4,20} = 0.21$ ,  $P = 0.927$ , day 26:  $F_{4,20} = 0.08$ ,  $P = 0.986$  and day 48:  $F_{4,20} =$   
 162  $0.28$ ,  $P = 0.891$ ) and no aggregations of microalgae and microplastics were observed during  
 163 the experiment. Cultures of *I. galbana* were prepared using seawater (35 psu), which was  
 164 filtered with 0.45  $\mu\text{m}$  aperture membranes and sterilised with UV light. Every day, each  
 165 mesocosm received 250 mL of  $\sim 2 \times 10^6$  cells mL<sup>-1</sup> of microalgae containing either 0  
 166 (control), 80 or 800  $\mu\text{g L}^{-1}$  of PLA or HDPE microplastics, equating to final densities in the  
 167 mesocosms of 2.5  $\mu\text{g L}^{-1}$  or 25  $\mu\text{g L}^{-1}$  (i.e. 250 mL diluted by the 8 L mesocosm  
 168 volume). During feeding the flow of water was stopped for two hours and air bubblers were

switched on in order to prevent anoxia and sedimentation of particulates. After this, the water flow in the mesocosms was resumed, replacing each mesocosm with clean seawater. The 2 hour daily exposure was chosen because in aquatic habitats, intermittent (as opposed to constant) exposure of contaminants is more likely to occur and, therefore, may be more environmentally relevant<sup>30,31</sup>. The experiment ran for 50 days, from the 26<sup>th</sup> of August until the 14<sup>th</sup> of October 2014. During this period the mean ( $\pm$  S.E.M) temperature of the water in the mesocosms was  $15.4 \pm 1.2$ .

## 2.2. Filtration rates of *M. edulis* and *O. edulis*

After 50 days, filtration rates were assessed by removing a single, randomly selected individual mussel or oyster from each mesocosm and holding them in separate 500 mL glass beakers with clean seawater each containing  $4 \times 10^3$  cells of *I. galabana* mL<sup>-1</sup>. Samples of 5 mL were taken after 0, 30, and 60 minutes and suspended algal cells were counted using a coulter counter. Tissue from each replicate was frozen at -20°C and later the dry biomass of each individual was determined by drying at 60°C for 24 h and weighing to the nearest  $\mu$ g to account for body mass. Filtration rates are expressed as the number of cells filtered mg<sup>-1</sup> of dry biomass h<sup>-1</sup>.

## 2.3. Porewater nutrients; ammonium, nitrate and nitrite

Porewater samples were collected using Rhizon<sup>TM</sup> membranes (Rhizosphere Research Products B.V., The Netherlands) inserted into the sampling ports of the mesocosms. This allowed water to be sampled at the surface (1 cm above the sediment), sediment-water interface (0 cm) and at 1 and 4 cm depths in the sediment. The flow of seawater into mesocosms was stopped and porewater was drawn by attaching a needle to each Rhizon<sup>TM</sup> membrane collecting 10 mL of water directly into sterile vacuum tubes (BD Vacutainer<sup>®</sup>).

Surface water was sampled a second and third time (at 30 minute intervals) to estimate nutrient fluxes. The water samples were stored in the vacuum tubes at 4°C prior to measuring concentrations of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) using a Lachat Quick-Chem 8000 flow injection autoanalyser with Lachat methods 31-107-06-1-B ( $\text{NH}_4^+$ ) and 31-107-04-1-A ( $\text{NO}_2^-$  and  $\text{NO}_3^-$  nitrate and nitrite). Porewater nutrient concentrations were adjusted for sediment porosity and standardised to dry bulk density. Pools of nutrients were calculated within the depth profile by integrating linear porewater concentration gradients, corrected for porosity, down to 4 cm depth. Concentrations of nitrate and nitrite were too minute (i.e. below the detection limit of  $\sim 0.01 \text{ mg L}^{-1}$ ) to be measured with confidence and were omitted from further analysis.

#### *2.4. Microalgal biomass on sediment surface*

A benthic fluorometer (BenthosTorch, bbe-Moldaenke GmbH, Schwentinental, Germany<sup>32</sup>) was used to estimate the biomass of diatoms and cyanobacteria on the sediment surface. Measurements were taken after 48 days, before any disturbance caused by other sampling activities. The BenthosTorch was placed on the surface at three random locations and averaged to serve as a single replicate measurement per mesocosm. Measurements are expressed in  $\mu\text{g biomass cm}^{-2}$ . Previous use of the BenthosTorch on similar sediment mesocosms found it to mirror the patterns of chlorophyll-a extraction using solvents<sup>33</sup>.

#### *2.5. Infaunal assemblages in the sediment*

Finally, all sediment was removed from each mesocosm and sieved separately through a 500  $\mu\text{m}$  mesh to retain macrofauna, which were placed into containers and topped up with 5% formalin and later enumerated and identified in the laboratory using Hayward and Ryland (1995)<sup>34</sup> as a key. Individuals were identified to species level where possible, and the number

of taxa ( $R$ ), the total number of individuals ( $N$ ) and Shannon-Wiener diversity ( $H'$ ) (with  $e$  as the base) were calculated as alpha-diversity measurements.

## 2.6. Statistical data analyses

Statistical analysis was done using the R environment (R v3.2.3; R core team 2015). The data were screened for normality (q-q plots, and Shapiro-Wilk tests) and homogeneity of variance (Levene's test, using the *car* (v2.1-2) package<sup>35</sup>) to ascertain assumptions for ANOVA. Transformation of some data was necessary to enable them to conform to these assumptions (specific transformations are stated in the results). Data were analysed separately for each of the two experiments (i.e. *M. edulis* and *O. edulis* were not compared in the statistical analyses). Since the design was asymmetrical (i.e. having a single control group for the two factors "Plastic" and "Dose"), the data were analysed by using the mean squares from two independent ANOVAs<sup>36</sup> (see Green et al., 2016<sup>33</sup> for more details on calculations). Briefly, this included partitioning of the variance by calculating: (1) one-way ANOVA with all treatments as separate levels ( $a=5$ ,  $n=5$ ,  $N=25$ ); and (2) a full-factorial two-way ANOVA of "Plastic" by "Dose" without the control ( $a=2$ ,  $b=2$ ,  $n=5$ ,  $N=20$ ). The residuals of the 1<sup>st</sup> ANOVA were used to assess differences between the levels within the 2<sup>nd</sup> ANOVA, allowing the variation associated with controls and that of the other treatments to be distinguished ("C vs. O"), which is contrasted with one degree of freedom<sup>36</sup>. When a significant effect in the "C vs. O" contrast was found Dunnett's test was used to contrast the control versus each level of the significant term using the *multcomp* (v1.4-6) package<sup>37</sup>. Pairwise comparisons for the factors in ANOVA (2) were computed using Tukey HSD tests when the main terms were significant. Statistical significance was assumed at  $\alpha = 0.05$ . Differences in invertebrate assemblage structure among treatments were compared using a two-factor permutational ANOVA based on Bray-Curtis dissimilarities of square root

transformed data with 9999 permutations under the reduced model using Type I sum of squares (SS) using PERMANOVA+ add-on (PRIMER-E Ltd. Plymouth, UK). The asymmetrical analyses were achieved by fitting each main effect (“Plastic” and “Dose”) in turn with a Type I (sequential) SS model, then swapping the order of the terms and combining the results of the two analyses<sup>38</sup>. When a factor was significant, contrasts were used to determine the specific differences. Results of the PERMANOVA were visualised with 2-dimensional ordination using canonical analysis of principal coordinates (CAP)<sup>39</sup>. Where assemblage structures differed, SIMPER analysis was used to quantify the contribution of different taxa to dissimilarities between treatments.

### 3. Results

#### 3.1. Effects of microplastics on the filtration rates of bivalves

Two mussels died during the experiment and were removed. There were no oyster mortalities. When exposed to  $25 \mu\text{g L}^{-1}$  of PLA or HDPE microplastics, *M. edulis* filtered ~2.4 times less microalgae (*I. galbana*) per hour than when exposed to none of the experimental microplastics (Figure 1a, Table S3, Dunnett's *Control vs 25  $\mu\text{g L}^{-1}$* :  $t=2.42$ ,  $P=0.045$ ). There was no effect of  $2.5 \mu\text{g L}^{-1}$  of either type of microplastic on the filtration of *M. edulis*. On the contrary, *O. edulis* in the control mesocosms filtered ~7.5 times less microalgae than those in mesocosms with any type or density of microplastic (Figure 1b, Table S3, Dunnett's *Control vs 25  $\mu\text{g L}^{-1}$* :  $t=-3.09$ ,  $P=0.011$ , *Control vs 2.5  $\mu\text{g L}^{-1}$* :  $t=-2.74$ ,  $P=0.024$ , *Control vs PLA*:  $t=-2.51$ ,  $P=0.038$ , *Control vs HDPE*:  $t=-2.74$ ,  $P=0.024$ ) compared to when not exposed to microplastics.

#### 3.2. Effects of microplastics on ammonium in sediment porewater

Concentrations of ammonium increased with depth in the sediment in all mesocosms (Figure 2). Sediment with *M. edulis* had no significantly different ammonium pools and ammonium

flux from the surface and was not significantly different between microplastic treatments (Table 1 and S4). Sediment with *O. edulis*, however, contained ~1.8 times more ammonium when no experimental microplastics were present compared with those dosed with either type of microplastic at both densities (Table S4, Dunnett's *Control vs PLA*:  $t=2.63$ ,  $P = 0.030$ ; *Control vs HDPE*:  $t=2.94$ ,  $P=0.015$ ). In addition, ammonium fluxes from the sediment into the water column were significantly different in mesocosms with *O. edulis* dosed with microplastics than in controls (Tables 1 and S4), however, *post-hoc* tests were unable to determine further significant differences.

### 3.3. Effects of microplastics on the microphytobenthos

The biomass of diatoms was not significantly different between the microplastic treatments for sediments with *M. edulis* or *O. edulis* (Table S4). The biomass of cyanobacteria, however, was significantly less in sediments which contained microplastics with *O. edulis* (Table S4) (but not those with *M. edulis*, Figure 3a), and was ~2 times greater in the controls than in mesocosms dosed with either type or density of microplastics (Figure 3b, Dunnett's *Control vs 25  $\mu\text{g L}^{-1}$  PLA*:  $t=4.77$ ,  $P<0.001$ ; *Control vs 2.5  $\mu\text{g L}^{-1}$  PLA*:  $t=3.91$ ,  $P=0.003$ ; *Control vs 25  $\mu\text{g L}^{-1}$  HDPE*:  $t=4.31$ ,  $P=0.001$ ; *Control vs 2.5  $\mu\text{g L}^{-1}$  HDPE*:  $t=3.05$ ,  $P=0.022$ ).

### 3.4. Effects of microplastics on infaunal assemblages

There were no significant differences between the structure of infaunal invertebrate assemblages (Figure 4a, Table S5), the diversity indices (Figure 5a, Table S6) nor the abundance of individual taxa (Table S6) in sediments with *M. edulis*. Sediments with *O. edulis*, however, had significantly different assemblage structures in treatments dosed with microplastics, at any density or type of plastic compared to controls (Figure 4b, Table S5) and there were several differences in dominance (Table S7). Although species richness and

total abundance did not differ significantly (Table S6), the Shannon-Wiener index ( $H'$ ) was ~2 times greater in controls than in mesocosms dosed with  $25 \mu\text{g L}^{-1}$  of HDPE microplastics (Figure 5b, Table S6, Dunnett's  $\text{Control vs } 25 \mu\text{g L}^{-1} \text{ HDPE}$ :  $t=0.14$ ,  $P=0.004$ ). There was a ~3 times greater abundance of *Eteone picta* polychaetes present in sediments not dosed with experimental microplastics than in treatments that received microplastics of either type (Figure 6a, Table S6, Dunnett's  $\text{Control vs } 25 \mu\text{g L}^{-1} \text{ PLA}$ :  $t=3.53$ ,  $P=0.008$ ;  $\text{Control vs } 2.5 \mu\text{g L}^{-1} \text{ PLA}$ :  $t=3.99$ ,  $P=0.002$ ;  $\text{Control vs } 25 \mu\text{g L}^{-1} \text{ HDPE}$ :  $t=4.27$ ,  $P=0.001$ ;  $\text{Control vs } 2.5 \mu\text{g L}^{-1} \text{ HDPE}$ :  $t=4.83$ ,  $P<0.001$ ). On the contrary, sediments in the controls had ~1.9 times fewer *Tubificoides benedii* oligochaetes than those dosed with  $25 \mu\text{g L}^{-1}$  of either type of microplastic (Figure 6b, Table S6, Dunnett's  $\text{Control vs } 25 \mu\text{g L}^{-1}$ :  $t=-3.27$ ,  $P=0.007$ ). There were also ~2.6 times more *Lineus longissimus* nemerteans in sediments when exposed to  $25 \mu\text{g L}^{-1}$  of PLA than in those exposed to  $2.5 \mu\text{g L}^{-1}$  of PLA or no microplastics (Figure 6c, Table S6, Tukey's HSD  $2.5 \mu\text{g L}^{-1} \text{ PLA vs } 25 \mu\text{g L}^{-1} \text{ PLA}$ :  $P=0.026$ , Dunnett's  $\text{Control vs } 25 \mu\text{g L}^{-1} \text{ PLA}$ :  $t=-2.66$ ,  $P=0.049$ ).

#### 4. Discussion

*Mytilus edulis* and *Ostrea edulis* responded differently to contamination with microplastics. The blue mussels filtered fewer algal cells  $\text{h}^{-1}$  when exposed to  $25 \mu\text{g L}^{-1}$  of PLA or HDPE microplastics. This supports findings of Wegner et al. (2012)<sup>40</sup> who found decreasing filtration rates with increasing concentrations (constant exposure of 0.1 - 0.3 g) of polystyrene nanoplastics (30 nm), but is in contrast with Browne et al., (2008)<sup>9</sup> which found no effect of constant exposure of 0.51 g of 3.0 or 9.6  $\mu\text{m}$  polystyrene microbeads on the filtration rates of *M. edulis* after 48 days. On the contrary, *O. edulis* exposed for 2 hours per day to 2.5 or  $25 \mu\text{g L}^{-1}$  of PLA or HDPE microplastics filtered more algae  $\text{h}^{-1}$  than when exposed to no microplastics. This is similar to another recent experiment, which found an increase in filtration rates of another species of oyster, *Crassostrea gigas*, in response to



constant exposure of  $23 \mu\text{g L}^{-1}$  of  $6 \mu\text{m}$  polystyrene microplastics<sup>12</sup>. From this selection of studies, albeit small, there is a pattern emerging suggesting a trend that mussels filter less and oysters filter more in response to plastic particles. More research is needed, however, to determine whether responses of filtration rates are generally applicable for bivalves in response to microplastics across different environmental contexts and with different polymer types.

Overall, the net filtration rates (corrected for dry weight of animal tissue) were greater for *M. edulis* than for *O. edulis*. Others have also found greater filtration rates (corrected for weight) in mussels than in oysters<sup>41,42</sup>. This could be because the microalgal concentrations in the filtration measurements were at  $4000 \text{ cells mL}^{-1}$  and *M. edulis* reaches optimum filtration rates at 2000 and  $6000 \text{ cells mL}^{-1}$ , whilst *O. edulis* requires concentrations an order of magnitude greater than this for optimum filtration rates to be reached<sup>44</sup>. Different bivalves may use different strategies when coping with an increase in particles (which would have occurred with the addition of microplastics). For example, under increased microalgae concentrations, mussels often decrease their filtration rates in order to maintain a constant consumption rate, whilst oysters increase theirs, along with their production of pseudofaeces<sup>45</sup>. Due to their importance in benthic-pelagic coupling, such alterations to filtration rates could lead to cascading effects on nutrient cycling and primary productivity in sedimentary habitats. In the current study, this occurred in the oyster-dominated mesocosms. The pool and flux of ammonium was less in the sediment pore-water with *O. edulis* exposed to microplastics. Although more research is required to ascertain the mechanisms to account for this result, it is possible that microbially-mediated processes which control the production (ammonification) and reduction (nitrification and denitrification) of ammonium were altered by the microplastics. Likely in response to there being less ammonium in the porewater, there was also less biomass of cyanobacteria. In a similar outdoor mesocosm experiment, PLA,

344 HDPE or PVC microplastics (2% of wet sediment weight) directly added to sandy sediment  
 345 led to reductions in the biomass of benthic diatoms, but not of cyanobacteria<sup>33</sup>. This  
 346 difference could be due to the grain size of the sediment. Cyanobacteria are less able to build  
 347 stable microbial mats on fine (muddy) sediment than they are on coarse (sandy) sediment,  
 348 whilst diatoms are stable on muddy sediment<sup>46</sup>. Nano- or micro- plastics have also been  
 349 found to reduce the productivity of other primary producers. For example, Besseling et al.  
 350 (2014)<sup>47</sup> found that nanoplastics reduced growth of green algae and overall chlorophyll  
 351 concentrations in laboratory microcosm experiments, and in another study Bhattacharya et al.  
 352 (2010)<sup>48</sup> reported a reduction of photosynthesis by microalgae. Cyanobacteria are key  
 353 primary producers in sedimentary systems<sup>49</sup>, vital in food-web dynamics<sup>50,51</sup>. Together with  
 354 euglenids and diatoms, they can supply up to 45% of the organic budget of an estuary<sup>14</sup> and  
 355 are important for stabilising sediments<sup>52</sup>. Decreases in the biomass of primary producers  
 356 (including cyanobacteria) could, therefore, induce cascading impacts on biodiversity and  
 357 ecosystem services<sup>53</sup>.

358 In the oyster-dominated mesocosms, perhaps in response to the decrease in cyanobacteria,  
 359 invertebrate assemblage structure was different in all treatments exposed to microplastics  
 360 compared with controls. Although species richness and the total abundance of infauna were  
 361 not affected by microplastics, assemblages in *O. edulis* treatments exposed to 25  $\mu\text{g L}^{-1}$  of  
 362 HDPE had lower Shannon-Weiner diversity indices, indicating that assemblages were more  
 363 homogeneous compared with controls. These differences were mostly caused by a greater  
 364 dominance of oligochaetes, *Tubificoides benedii*, in all treatments with microplastics (which  
 365 contributed ~30% of the difference between controls and each microplastic treatment, Table  
 366 S7). Oligochaetes typically respond opportunistically to stressors and have long been  
 367 considered as indicators of pollution in marine<sup>54</sup> and freshwater<sup>55</sup> systems. Mesocosms dosed  
 368 with 25  $\mu\text{g L}^{-1}$  of PLA or HDPE microplastics also had less *E. picta* (paddle worms). A

369 reduction in the abundance of paddle worms, which are often specialist predators, has been  
370 found in response to other stressors, such as nutrient enrichment, in sedimentary systems<sup>56</sup>.  
371 Additionally, the abundance of *Lineus longissimus* (bootlace worms) was greater in  
372 treatments with 25  $\mu\text{g L}^{-1}$  PLA microplastics compared to those with 2.5  $\mu\text{g L}^{-1}$  or no  
373 microplastics. These worms are also a potential indicator species of pollution<sup>57</sup>. The  
374 dominance of opportunistic species, suggests a simplification of the food web in response to  
375 high levels of microplastic contamination.

376 Interestingly, there were no measurable effects of microplastic exposure on infaunal  
377 invertebrate assemblages in the sediments with *M. edulis* mussels. This may be due to the  
378 different effects of microplastics on filtration activity of the bivalves. For example, the  
379 increase in filtration rates of *O. edulis* were likely accompanied by an increase in the  
380 production of pseudofaeces. Particles rejected by bivalves as pseudofaeces are embedded in  
381 mucous and sink<sup>58</sup>, possibly increasing the availability of microplastics to the *O. edulis*  
382 benthic communities. It is also possible that greater habitat complexity, due to the presence of  
383 more shells in the mussel experiment, mitigated any effects of microplastics on ecosystem  
384 functioning or on assemblages compared to in the oyster experiment. Habitat structure can  
385 influence movement and resource utilisation of organisms and can alter the direct and indirect  
386 interactions between species<sup>59</sup>. In order to fully understand the role of different ecosystem  
387 engineers in mediating or exacerbating the effect of microplastics, experiments comparing  
388 community level effects with and without ecosystem engineers present are needed.

389 Regardless of the mechanisms, this study shows that microplastics may affect ecosystem  
390 functioning and biodiversity but, as has been found for other pollutants<sup>60</sup>, such effects are  
391 context-dependent.

392 Manipulation of microplastics in field conditions would be difficult and would pollute,  
393 therefore, the use of an outdoor mesocosm system, with natural seawater and weather

conditions and recruitment of meio- and micro-organisms, provided an ideal compromise between the highly controlled conditions of a laboratory experiment and the realism of a field experiment. The extrapolation of results from any mesocosm experiment should, however, proceed with caution since the assemblages represented in the mesocosms are simplified, compared to the field. For example, pelagic larval recruitment<sup>61</sup> and other complex processes involving larger organisms (such as fish), are excluded from the cores<sup>62</sup>. Regardless, semi-field experiments, such as those using intact cores are a useful technique for evaluating the effects of stressors on infaunal communities<sup>63,64,65</sup> and they have been found to produce ecologically relevant data<sup>66,67</sup>. A similar mesocosm experiment using intact cores and *Ostrea edulis* (in vegetated, sandy rather than muddy sediment) also found alterations to assemblage structure and a reduction in diversity, specifically with less isopods, amphipods and periwinkle snails after 60 days of exposure to 80  $\mu\text{g L}^{-1}$  of HDPE or PLA microplastics<sup>13</sup>. Together these two pioneering studies indicate that microplastics could alter benthic assemblages in *O. edulis*-dominated sedimentary habitats if they are repeatedly exposed (even for just 2 hours per day) to concentrations as high as 2.5, 25 or 80  $\mu\text{g L}^{-1}$ . Globally, oyster populations are under threat, and due to overfishing, parasites and disease<sup>68</sup>, 85% of oyster reefs have been lost world-wide<sup>69</sup>. Declines in oyster reefs are cause for concern, not only because they provide protein sources and support the fishing industry, but also because of their role in the provision of ecosystem services in the coastal zone<sup>70</sup>. The current study suggests that microplastics may represent an additional pressure to the organisms living in these already threatened habitats.

#### *Wider implications and recommendations*

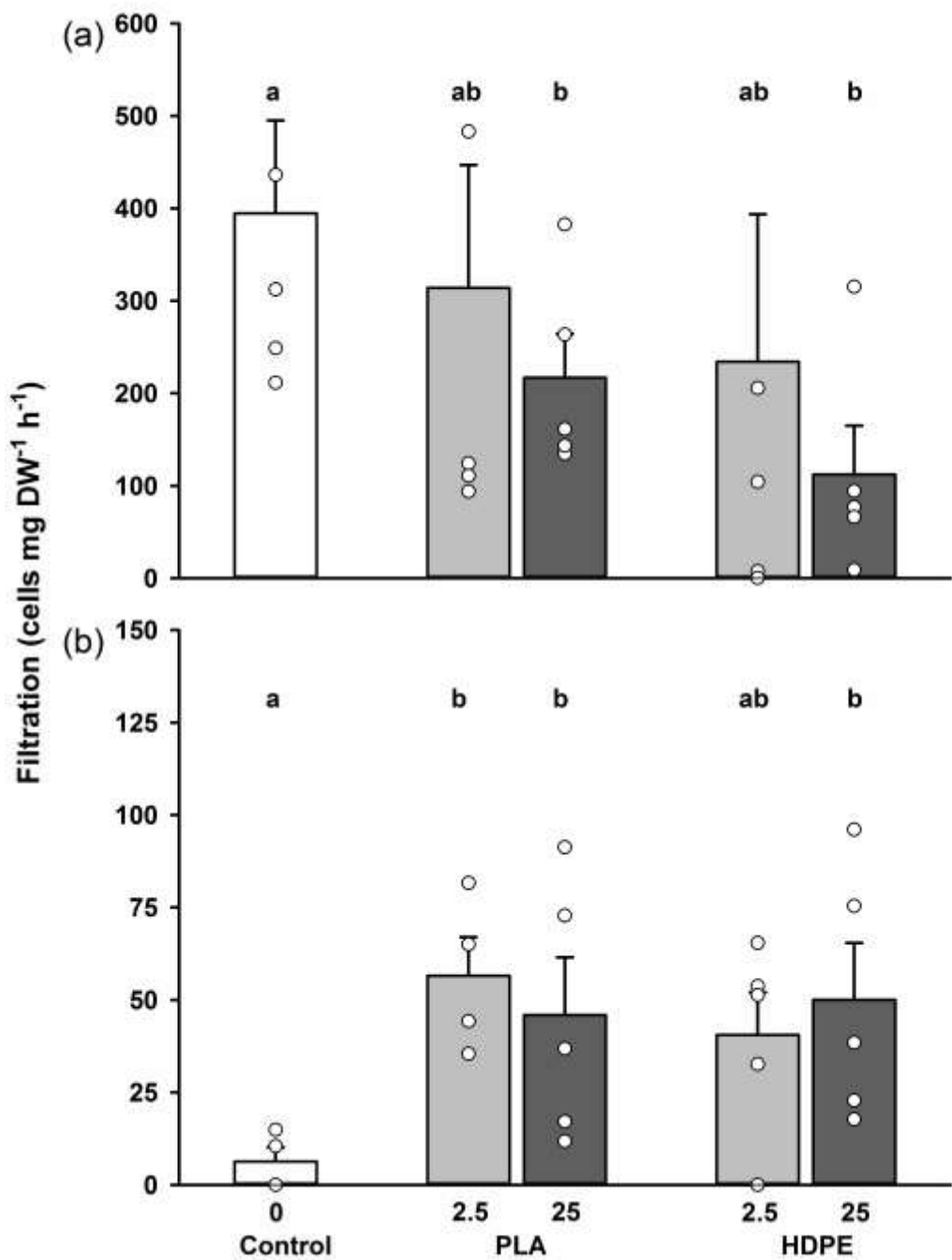
Since microplastics composed of PLA did not rapidly decompose, they caused many of the same impacts as HDPE to *M. edulis* and *O. edulis* and the sediment they inhabit. This,

combined with evidence from other studies<sup>13,33,71</sup>, supports recommendations that the term "biodegradable" should be redefined to ensure that material, such as PLA, that apparently does not rapidly and fully degrade in aquatic habitats does not enter drainage systems as microscale particles such as microbeads<sup>72</sup> or enter the environment as larger litter.

Alterations to invertebrate assemblages in oyster-dominated sediment was detected at just 2.5  $\mu\text{g L}^{-1}$ , although this dose is high, it is conservative compared to other recent experimental studies e.g. 1250 - 25000<sup>73</sup>  $\mu\text{g L}^{-1}$  and 200 - 4800<sup>22</sup>  $\mu\text{g L}^{-1}$ . It is also much lower than current levels found in some heavily contaminated coasts, for example,  $\sim 4500$   $\mu\text{g L}^{-1}$  in Korea<sup>6</sup>.

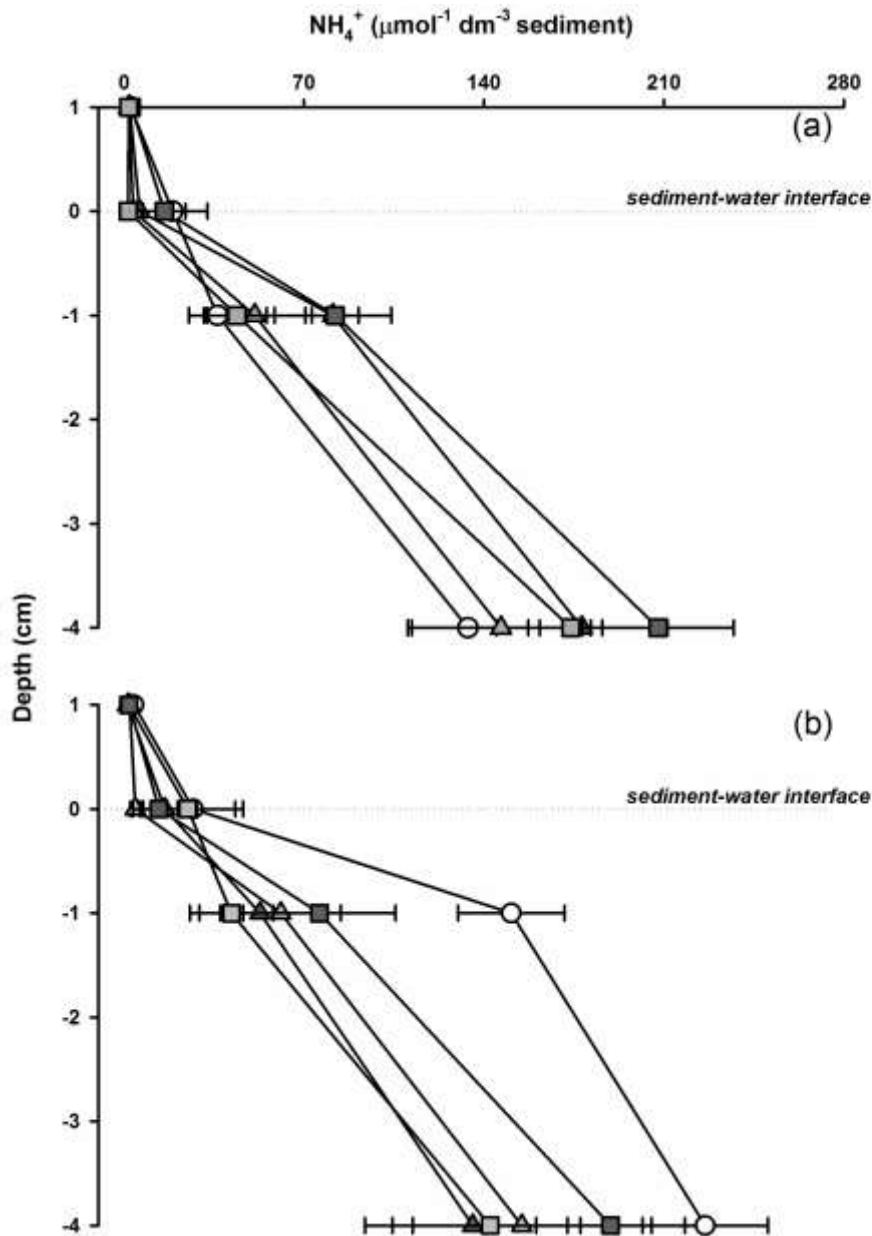
Current plankton net sampling techniques, however, typically have a lower size limit of  $\sim 300$   $\mu\text{m}$ , therefore underestimating current densities of microplastics, possibly by between 3 and 6 orders of magnitude when compared to a 10  $\mu\text{m}$  mesh<sup>74</sup>. Also, given that the cumulative input of plastic waste is expected to increase in the coming decades<sup>27</sup> and that the fragmentation of macroplastic litter already present in the environment will continue, concentrations of microplastics are expected to increase<sup>75</sup>. In the current study, effects on ammonium concentrations and biomass of cyanobacteria occurred at just 2.5  $\mu\text{g L}^{-1}$ . Wider effects of microplastics on nutrient cycling and invertebrate assemblages could, therefore, already be occurring in heavily contaminated oyster-dominated habitats, however more research, including mensurative studies, are needed to ascertain this.

The current study provides ecologically relevant data on the effects of contamination by microplastic of different polymers, focusing on assemblage-level effects and ecosystem functioning. Such data is currently still rare in the literature, but is vital in order to inform policy and prevent damage to ecosystems<sup>7</sup>. In order to fully assess the ecological impacts of microplastics, however, we also need to test their effects at low concentrations and using realistic mixtures of polymer types (as opposed to one type at a time).



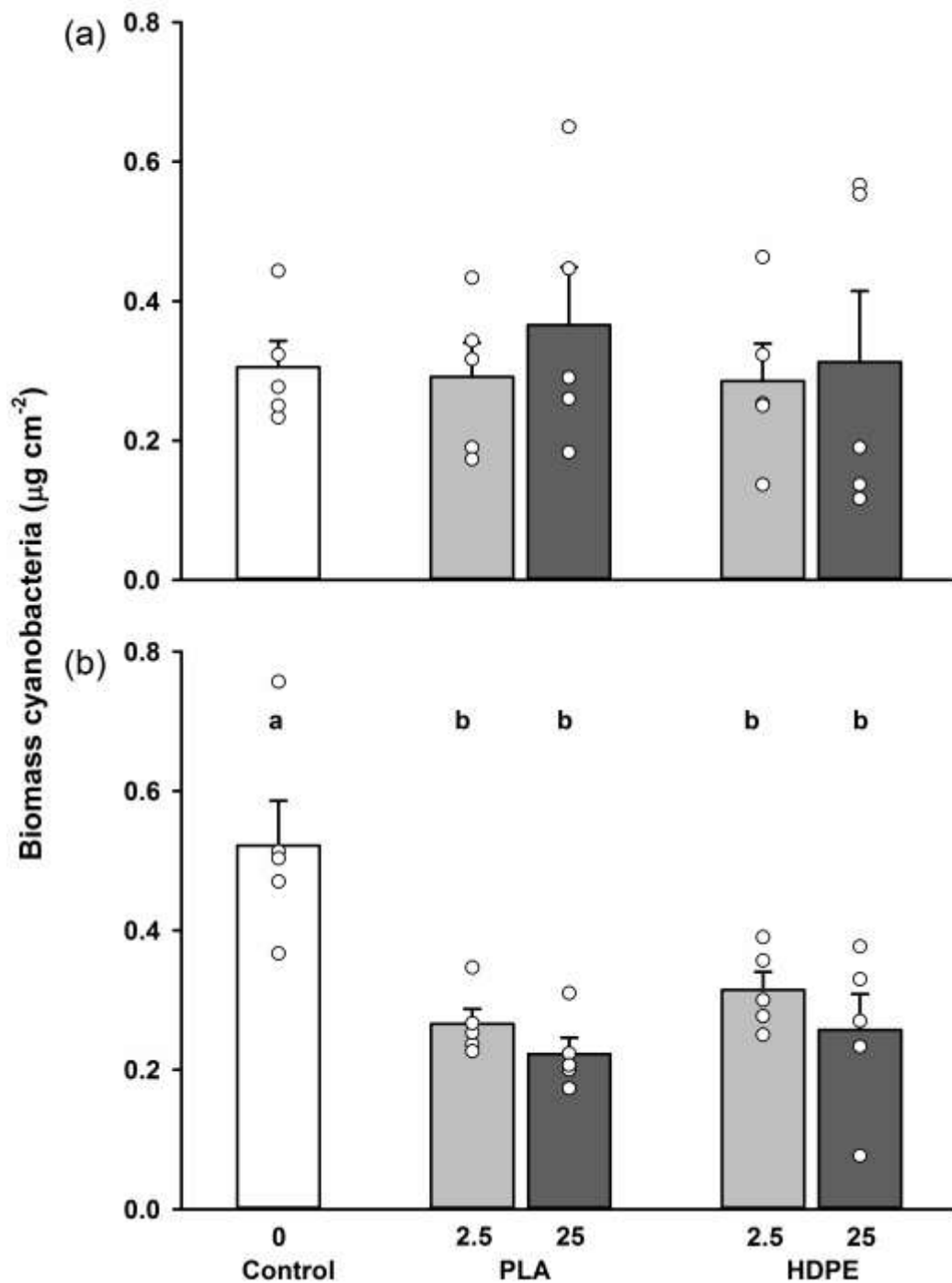
444

445 **Figure 1.** Filtration rates of (a) *M. edulis* and (b) *O. edulis* in mesocosms with 2.5 µg L<sup>-1</sup> or  
446 25 µg L<sup>-1</sup> of PLA or HDPE or with no microplastics (Control) after 50 days. Different letters  
447 indicate significant differences among treatments as determined by *post-hoc* comparisons or  
448 Dunnett's tests. Circles represent raw data, bars are means (± S.E.M.) with n = 5.



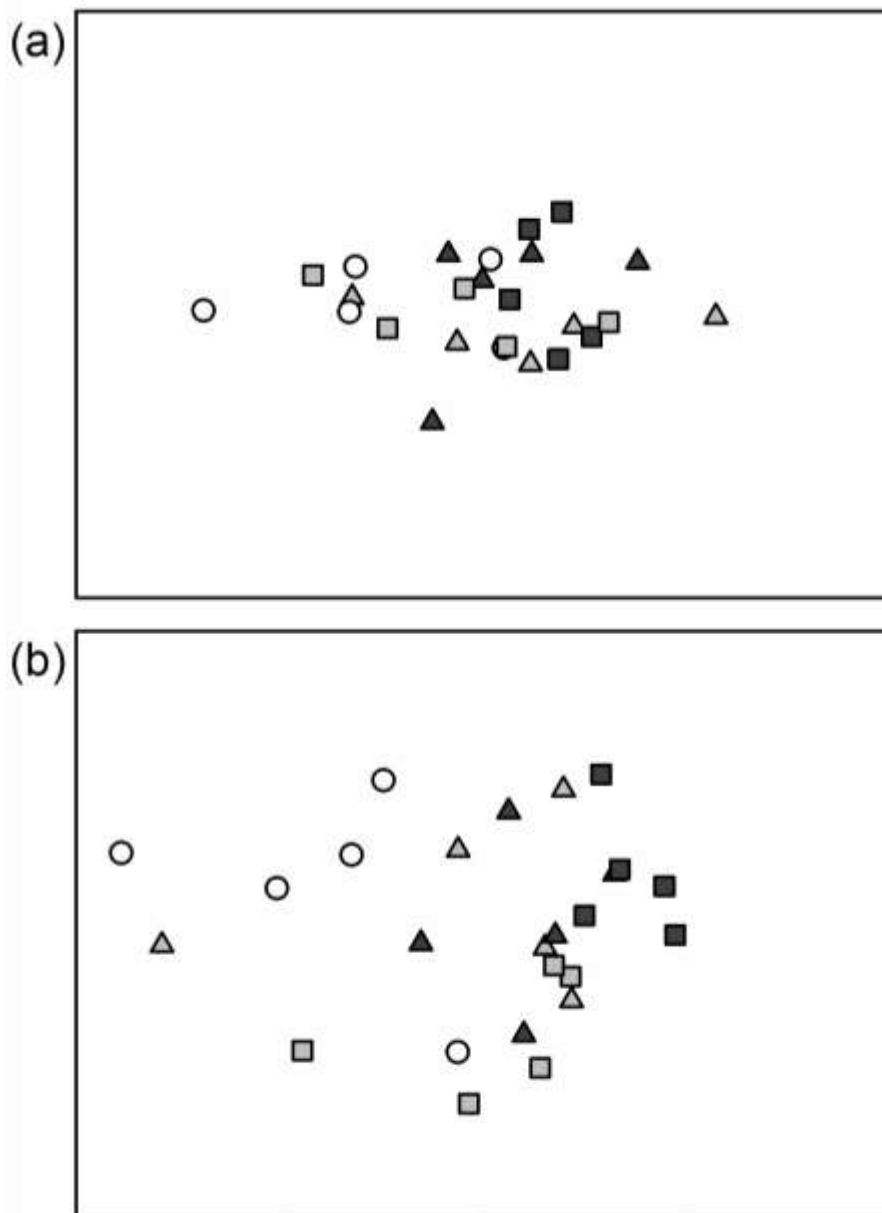
449

450 **Figure 2.** Concentrations of  $\text{NH}_4^+$  in mesocosms with (a) *M. edulis* or (b) *O. edulis* of surface  
 451 water (1 cm), the sediment-water interface (0 cm), and 1 & 4 cm into the sediment in  
 452 mesocosms with 2.5  $\mu\text{g L}^{-1}$  (△) or 25  $\mu\text{g L}^{-1}$  (▲) of PLA or 2.5  $\mu\text{g L}^{-1}$  (◻) or 25  $\mu\text{g L}^{-1}$  (◼)  
 453 of HDPE microplastics or no microplastics (Control = ○) after 50 days. Data are means (±  
 454 S.E.M.) with n = 5.



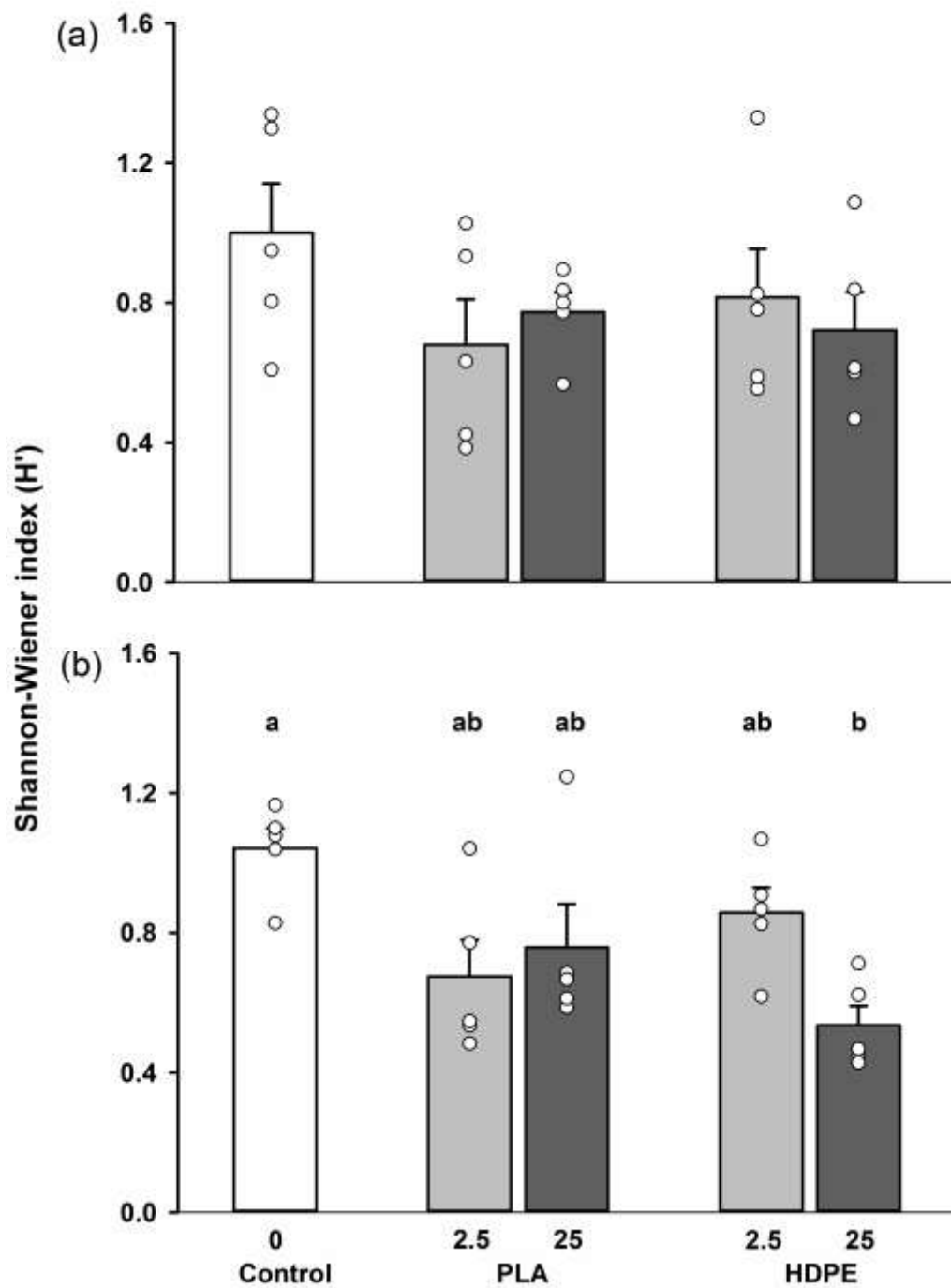
**Figure 3.** Biomass of cyanobacteria in mesocosms with (a) *M. edulis* or (b) *O. edulis* and 2.5 µg L<sup>-1</sup> or 25 µg L<sup>-1</sup> of PLA or HDPE or with no microplastics (Control) after 48 days. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data and bars are means (± S.E.M.) with n = 5.



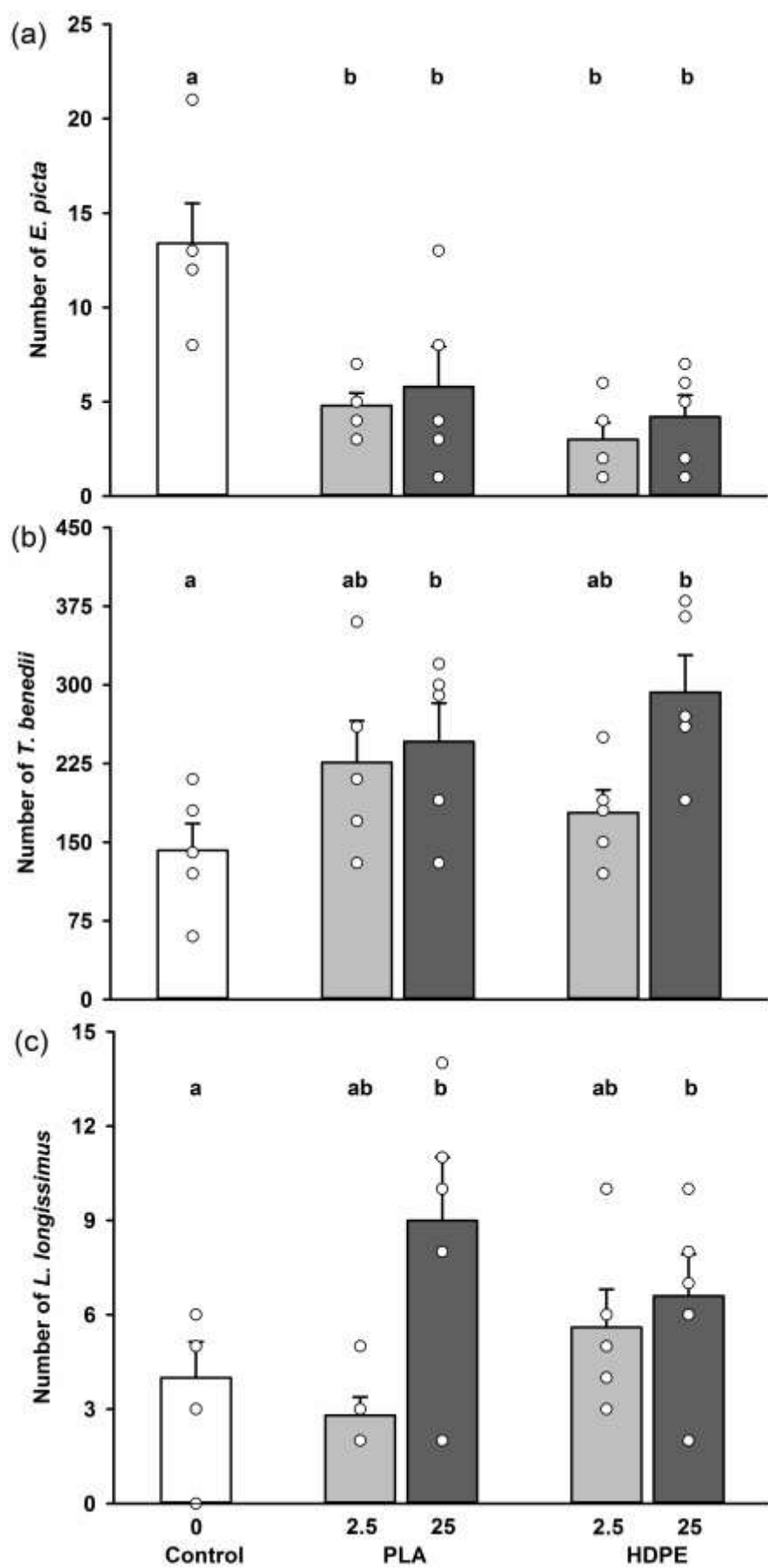


461

462 **Figure 4.** Canonical analysis of principal coordinates of square root transformed community  
 463 structure data in mesocosms with (a) *M. edulis* or (b) *O. edilis* and 2.5 µg L<sup>-1</sup> (▲) or 25 µg  
 464 L<sup>-1</sup> (▲) of PLA or 2.5 µg L<sup>-1</sup> (◻) or 25 µg L<sup>-1</sup> (◼) of HDPE microplastics or with no  
 465 microplastics (control = ○) after 50 days, n = 5.



**Figure 5.** Shannon Wiener diversity index in mesocosms with (a) mussels or (b) oysters and 2.5 µg L<sup>-1</sup> or 25 µg L<sup>-1</sup> of PLA or HDPE or with no microplastics (control) after 50 days. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (± S.E.M.) with n = 5.



473 **Figure 6.** Abundances of (a) *E. picta*, (b) *T. benedii* and (c) *L. longissimus* in oyster  
474 treatments with 2.5  $\mu\text{g L}^{-1}$  or 25  $\mu\text{g L}^{-1}$  of PLA or HDPE or with no microplastics (control)  
475 after 50 days. Data from *M. edulis* mesocosms are not shown. Different letters indicate  
476 significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's  
477 tests. Circles represent raw data, and bars are mean ( $\pm$  S.E.M.) with  $n = 5$ .

478 **Table 1.** Porewater ammonium pool ( $\mu\text{mol dm}^{-3}$ ) and flux ( $\mu\text{mol h}^{-1}$ ) in mesocosm sediment  
 479 after 50 days with *M. edulis* or *O. edulis* and no microplastics (control), or the two doses of  
 480 microplastics. Different superscript letters indicate significance between treatments. Data are  
 481 means ( $\pm$  S.E.M.) with n = 5.

		<i>M. edulis</i>		<i>O. edulis</i>	
		$\text{NH}_4^+$ pool	$\text{NH}_4^+$ flux	$\text{NH}_4^+$ pool	$\text{NH}_4^+$ flux
<b>Control</b>	<b>0 <math>\mu\text{g L}^{-1}</math></b>	436.89 $\pm$ 77.63 <sup>a</sup>	0.14 $\pm$ 3.99 <sup>a</sup>	669.23 $\pm$ 80.57 <sup>a</sup>	-57.18 $\pm$ 38.61 <sup>a</sup>
<b>PLA</b>	<b>2.5 <math>\mu\text{g L}^{-1}</math></b>	293.68 $\pm$ 33.29 <sup>a</sup>	-19.68 $\pm$ 13.98 <sup>a</sup>	359.52 $\pm$ 108.41 <sup>b</sup>	-19.88 $\pm$ 4.52 <sup>a</sup>
	<b>25 <math>\mu\text{g L}^{-1}</math></b>	493.00 $\pm$ 52.43 <sup>a</sup>	-25.58 $\pm$ 9.96 <sup>a</sup>	325.83 $\pm$ 54.25 <sup>b</sup>	2.34 $\pm$ 2.54 <sup>a</sup>
<b>HDPE</b>	<b>2.5 <math>\mu\text{g L}^{-1}</math></b>	326.25 $\pm$ 79.16 <sup>a</sup>	-3.28 $\pm$ 8.25 <sup>a</sup>	322.85 $\pm$ 69.08 <sup>b</sup>	-8.76 $\pm$ 2.38 <sup>a</sup>
	<b>25 <math>\mu\text{g L}^{-1}</math></b>	351.19 $\pm$ 24.21 <sup>a</sup>	-14.34 $\pm$ 6.56 <sup>a</sup>	450.64 $\pm$ 94.07 <sup>b</sup>	-10.42 $\pm$ 9.08 <sup>a</sup>

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## ASSOCIATED CONTENT

File name “Supporting Material.pdf” containing 10 pages (cover page included), containing 1 figure and 7 tables.

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## Author Contributions

D.S.G. conceived the idea and designed the experiment. D.S.G. and B.B. carried out the field and laboratory work and analysed the data. All authors contributed to writing the manuscript and all authors have given approval to the final version of the manuscript.

## Notes

The authors declare no competing financial interests.

## Funding Sources

This research was funded the Irish Research Council with a Postdoctoral Research Project Grant (GOIPD/2013/306) and the G.M. Williams Fund and Royal Society Research Grant (RG120432).

## Acknowledgements

The authors are very grateful to F. Glynn, B. McNamara and C. Guillaumot for assistance in the field, to E. Gorman for microalgal culturing and to S. Jiang for nutrient analysis. Thanks to G. Chapman and M. Anderson for advice on asymmetrical analyses. This research was funded by the Irish Research Council with a Postdoctoral Research Project Grant (GOIPD/2013/306) awarded to D.S.G., the G.M. Williams Fund and Royal Society Research Grant (RG120432) to N.E.O'C.

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## SUPPORTING INFORMATION

### **Microplastics affect the ecological functioning of an important biogenic habitat**

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**This file has 10 pages (cover page included), containing one figure and 7 tables as referred to in the main manuscript:**

Figure S1 (page S3): Schematic diagram of bucket as mesocosm.

Table S1 (page S4): Approximate density of microplastic particles per treatment estimated using a haemocytometer.

Table S2 (page S5): Microalgae cell counts estimated a haemocytometer.

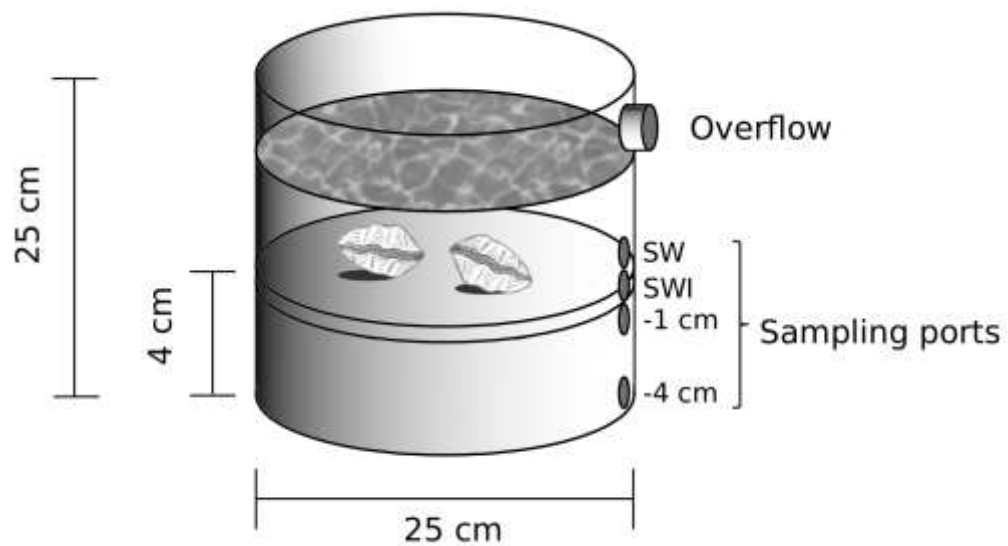
Table S3 (page S6): Asymmetric ANOVA results for filtration.

Table S4 (page S7): Asymmetric ANOVA results for nutrients and microalgal biomass.

Table S5 (page S8): Asymmetric permutational multivariate ANOVA results for assemblage structures

Table S6 (page S9): Asymmetric ANOVA results of number of taxa (R), total abundance (N), Shannon Wiener diversity (H') and abundances of *E. picta*, *T. benedii* and *L. longissimus*.

Table S7 (page S10): SIMPER results of benthic assemblages.



**Figure S1.** Diagram showing the design of the mesocosms with sampling ports for surface water (SW), the sediment-water interface (SWI) and for porewater at 1 and 4 cm into the sediment.

**Table S1.** Approximate number of microplastic particles per treatment ( $L^{-1}$ ), estimated using haemocytometer counts on water samples taken from mesocosms directly after dosing on days 1, 26 and 48 of the experiment.

Plastic	Dose ( $\mu g L^{-1}$ )	Day 1	Day 26	Day 48
Control	0	0	0	0
PLA	2.5	$260.42 \pm 125.43$	$156.25 \pm 45.54$	$138.89 \pm 45.35$
	25	$1406.25 \pm 193.48$	$1163.19 \pm 165.29$	$1319 \pm 189.99$
HDPE	2.5	$104.17 \pm 65.88$	$86.81 \pm 42.31$	$86.81 \pm 33.95$
	25	$937.50 \pm 213.48$	$815.97 \pm 80.44$	$763.89 \pm 126.88$

**Table S2.** Mean ( $\pm$ S.E.M, n = 5) number of cells of *Isochrysis galbana* ( $\text{mL}^{-1}$ ) estimated using haemocytometer counts in batches algal cultures for use in 2.5 or 25  $\mu\text{g L}^{-1}$  of PLA or HDPE microplastic treatments or in Controls (with no microplastics) on days 1, 26 and 48 of the experiment.

Plastic	Dose ( $\mu\text{g L}^{-1}$ )	Day 1	Day 26	Day 48
Control	0	$2.16 \times 10^6 \pm 2.37 \times 10^5$	$1.94 \times 10^6 \pm 1.37 \times 10^5$	$1.96 \times 10^6 \pm 2.31 \times 10^5$
PLA	2.5	$2.43 \times 10^6 \pm 6.01 \times 10^5$	$1.97 \times 10^6 \pm 3.30 \times 10^5$	$1.92 \times 10^6 \pm 2.22 \times 10^5$
	25	$2.45 \times 10^6 \pm 3.47 \times 10^5$	$1.90 \times 10^6 \pm 7.07 \times 10^4$	$2.07 \times 10^6 \pm 2.14 \times 10^5$
HDPE	2.5	$2.19 \times 10^6 \pm 2.59 \times 10^5$	$1.91 \times 10^6 \pm 2.82 \times 10^5$	$1.81 \times 10^6 \pm 1.72 \times 10^5$
	25	$2.52 \times 10^6 \pm 1.39 \times 10^5$	$1.80 \times 10^6 \pm 1.72 \times 10^5$	$2.16 \times 10^6 \pm 3.94 \times 10^5$

**Table S3.** Asymmetric ANOVA results of filtration of *M. edulis* and *O. edulis* after 50 days.

The term "One-way" has 4,20 degrees of freedom (numerator and denominator, respectively) and all other terms have 1,20 degrees of freedom. F ratios with P significant at  $\alpha = 0.05$  are indicated in **bold**.

<i>M. edulis</i>		
Source	F ratio	P value
One-way	1.62	0.330
C vs. O*	<b>4.58</b>	<b>0.045</b>
Plastic (P)	1.46	0.241
Dose (D)	0.37	0.548
P x D	0.06	0.805
<i>O. edulis</i>		
One-way	<b>3.20</b>	<b>0.038</b>
C vs. O	<b>11.63</b>	<b>0.003</b>
Plastic (P)	0.30	0.593
Dose (D)	0.00	0.958
P x D	0.86	0.366

\* C vs. O = contrast comparing the control versus all others

**Table S4.** Asymmetric ANOVA on pool ( $\mu\text{mol dm}^{-3}$ ) and flux ( $\mu\text{mol h}^{-1}$ ) of  $\text{NH}_4^+$ , and biomass ( $\mu\text{g cm}^{-2}$ ) of diatoms and cyanobacteria in the sediment after 48 days. The term "One-way" has 4,20 degrees of freedom (numerator and denominator, respectively) and all other terms have 1,20 degrees of freedom. Data are F ratios with P values (those significant at  $\alpha = 0.05$  are indicated in **bold**). In order to conform to the assumptions of normality, data for cyanobacteria were square-root transformed in the experiment with *O. edulis*.

<i>M. edulis</i>								
Source	$\text{NH}_4^+$ pool		$\text{NH}_4^+$ flux		Diatoms		Cyanobacteria	
	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
One-way	2.03	0.129	1.40	0.271	0.17	0.953	0.21	0.927
C vs. O	1.20	0.286	2.39	0.138	0.00	0.959	0.01	0.914
Plastic (P)	0.89	0.356	2.27	0.148	0.05	0.827	0.18	0.672
Dose (D)	3.76	0.067	0.85	0.367	0.49	0.491	0.54	0.469
P x D	2.27	0.147	0.08	0.782	0.12	0.727	0.12	0.736
<i>O. edulis</i>								
One-way	<b>3.05</b>	<b>0.041</b>	1.63	0.206	1.33	0.293	<b>7.23</b>	<b>0.001</b>
C vs. O	<b>10.7</b>	<b>0.004</b>	<b>5.74</b>	<b>0.027</b>	3.30	0.084	<b>25.71</b>	<b>&lt;0.001</b>
Plastic (P)	0.28	0.603	0.00	0.964	0.01	0.922	0.88	0.360
Dose (D)	0.32	0.579	0.33	0.573	0.94	0.344	2.25	0.149
P x D	0.94	0.345	0.44	0.513	1.07	0.313	0.08	0.780

**Table S5.** Asymmetric permutational multivariate ANOVA results for assemblage structures in sediments with *M. edulis* or *O. edulis* and 2.5 or 25  $\mu\text{g L}^{-1}$  of PLA or HDPE microplastics, or controls (C) with no microplastics after 50 days. When the factors "Plastic" or "Dose" were significant (at  $\alpha = 0.05$ , indicated in **bold**), contrasts were used to determine any differences among treatments between levels.

Source	Contrasts	d.f.*	<i>M. edulis</i>		<i>O. edulis</i>	
			F-value	P-value	F-value	P-value
One-way		4	0.93	0.500	1.83	0.088
Plastic (P)		2	1.10	0.350	<b>2.51</b>	<b>0.037</b>
	PLA vs. HDPE	1	-	-	0.32	0.781
	PLA vs. C	1	-	-	<b>3.61</b>	<b>0.028</b>
	HDPE vs. C	1	-	-	<b>3.84</b>	<b>0.018</b>
Dose (D)		2	1.21	0.277	<b>3.10</b>	<b>0.026</b>
	2.5 vs. 25	1	-	-	1.65	0.167
	2.5 vs. C	1	-	-	<b>3.00</b>	<b>0.043</b>
	25 vs. C	1	-	-	<b>4.72</b>	<b>0.015</b>
PxD		1	0.78	0.545	0.82	0.461

\*d.f. = degrees of freedom of nominator

**Table S6.** Asymmetric ANOVA results of number of taxa (R), total abundance (N), Shannon Wiener diversity (H') and abundances of *E. picta*, *T. benedii* and *L. longissimus* after 50 days. The term "One-way" has 4,20 d.f.'s and all other terms have 1,20 d.f.'s. (numerator and denominator, respectively) F ratios (F) with P significant at  $\alpha=0.05$  are indicated in **bold**. In order to conform to the assumptions of homogeneity of variance, R and N were square-root transformed for data from the *M. edulis* experiment.

<i>M. edulis</i>												
Source	R		N		H'		<i>E. picta</i>		<i>T. benedii</i>		<i>L. longissimus</i>	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
One-way	0.81	0.536	0.55	0.703	1.08	0.393	0.52	0.725	0.61	0.658	1.37	0.279
C vs. O	2.80	0.109	1.06	0.314	3.57	0.073	0.83	0.373	1.27	0.272	1.61	0.219
Plastic (P)	0.24	0.630	0.08	0.777	0.12	0.730	0.12	0.732	0.00	0.990	1.70	0.206
Dose (D)	0.18	0.674	0.20	0.659	0.00	1.000	0.56	0.465	0.11	0.740	0.37	0.548
P x D	0.00	0.950	0.84	0.370	0.62	0.439	0.56	0.465	1.07	0.313	1.79	0.196
<i>O. edulis</i>												
One-way	2.09	0.119	1.59	0.217	<b>4.84</b>	<b>0.007</b>	<b>7.36</b>	<b>0.001</b>	<b>3.25</b>	<b>0.033</b>	<b>3.24</b>	<b>0.033</b>
C vs. O	0.01	0.936	3.49	0.076	<b>11.95</b>	<b>0.003</b>	<b>27.67</b>	<b>&lt;0.001</b>	<b>6.61</b>	<b>0.018</b>	1.81	0.194
Plastic (P)	2.70	0.116	0.23	0.638	0.06	0.812	1.25	0.277	0.00	0.989	0.02	0.882
Dose (D)	1.63	0.216	2.02	0.171	1.88	0.185	0.52	0.478	4.28	0.052	3.81	0.065
P x D	4.03	0.058	0.61	0.45	<b>5.47</b>	<b>0.029</b>	0.00	0.948	2.12	0.161	<b>7.31</b>	<b>0.014</b>



**Table S7.** SIMPER analyses based on square-root transformed abundance data within the sediment from the *O. edulis* mesocosms with no microplastics (control = C) versus 2.5 µg L<sup>-1</sup> of PLA (2.5 PLA), 25 µg L<sup>-1</sup> of PLA (25 PLA), 2.5 µg L<sup>-1</sup> of HDPE (2.5 HDPE) or 25 µg L<sup>-1</sup> of HDPE (25 HDPE).

	Average abundance		Av.Diss <sup>*</sup>	Diss/SD <sup>**</sup>	Contrib % <sup>***</sup>	Cum.% <sup>****</sup>
Taxon	C vs. 2.5 PLA					
<i>T. benedii</i>	12.74	16.71	8.43	1.44	29.93	29.93
<i>Corophium</i> sp.	2.89	4.52	5.22	1.49	18.52	48.45
Spionidae	4.03	3.22	2.61	1.37	9.28	57.73
<i>Glycera</i> sp.	2.87	2.82	2.34	1.38	8.32	66.05
<i>E. picta</i>	3.08	2.27	1.92	1.62	6.82	72.86
<i>L. longissimus</i>	2.2	1.48	1.89	1.23	6.7	79.57
<i>Hydrobia</i> sp.	1.2	0.51	1.63	1.2	5.79	85.36
C vs. 25 PLA						
<i>T. benedii</i>	12.74	15.56	7.04	1.43	26.92	26.92
<i>Corophium</i> sp.	2.89	2.99	4.03	1.4	15.4	42.32
<i>Glycera</i> sp.	2.87	2.8	2.9	1.33	11.07	53.39
Spionidae	4.03	3.92	2.89	1.27	11.04	64.43
<i>E. picta</i>	3.08	2.53	1.95	1.4	7.46	71.89
<i>L. longissimus</i>	2.2	2.52	1.78	1.29	6.78	78.68
<i>Hydrobia</i> sp.	1.2	0.6	1.73	1.24	6.6	85.28
C vs. 2.5 HDPE						
<i>T. benedii</i>	12.74	14.63	7.04	1.27	28.2	28.2
<i>Corophium</i> sp.	2.89	3.71	3.66	1.38	14.68	42.87
Spionidae	4.03	4.28	2.41	1.52	9.65	52.52
<i>Glycera</i> sp.	2.87	2.4	2.38	1.44	9.52	62.04
<i>E. picta</i>	3.08	2.11	2.16	1.5	8.64	70.67
<i>Hydrobia</i> sp.	1.2	0.62	1.59	1.19	6.37	77.05
<i>L. longissimus</i>	2.2	2.47	1.54	1.25	6.18	83.23
C vs. 25 HDPE						
<i>T. benedii</i>	12.74	16.81	8.87	1.38	32.39	32.39
<i>Corophium</i> sp.	2.89	2.53	4.08	1.29	14.91	47.31
Spionidae	4.03	3.04	2.83	1.34	10.34	57.65
<i>Glycera</i> sp.	2.87	3.4	2.71	1.34	9.91	67.55
<i>E. picta</i>	3.08	2.29	1.94	1.41	7.09	74.65
<i>Hydrobia</i> sp.	1.2	0.5	1.82	1.21	6.65	81.3
<i>L. longissimus</i>	2.2	2.23	1.49	1.22	5.43	86.74

\* Av. Diss. = average “absolute” contribution of taxon to total dissimilarity between pairs based on Bray-Curtis dissimilarities

\*\* Diss/SD = ratio of average contribution to dissimilarity and the standard deviation among all contribution across all pairs of samples

\*\*\* Contrib % = contribution of taxon in % to dissimilarity between the two samples

\*\*\*\* Cum. % = cumulative percentage of contribution of taxon to the dissimilarity between the two sample.

